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Abstract

***Background*—**Patients with Chronic Granulomatous Disease (CGD) suffer immunodeficiency due to defects in the phagocyte NADPH oxidase (NOX2) and concomitant reduction in reactive oxygen intermediates. This may result in a reduction in atherosclerotic injury.

***Methods and Results*—**We prospectively assessed the prevalence of cardiovascular risk factors, biomarkers of inflammation and neutrophil activation, and the presence of MRI and CT quantified subclinical atherosclerosis in the carotid and coronary arteries of 41 CGD patients and 25 healthy controls in the same age range. Uni- and multivariable associations between risk factors, inflammatory markers and atherosclerosis burden were assessed. CGD patients had significant elevations in traditional risk factors and inflammatory markers compared with controls, including; hypertension, hsCRP, oxidized LDL, and low HDL. Despite this, CGD patients had a 22% lower internal carotid artery wall volume compared with controls (361.3 ± 76.4 mm^3 vs. 463.5 ± 104.7 mm^3, $p<0.001$). This difference was comparable in $p47^{phox}$ and $gp91^{phox}$ deficient subtypes of CGD, and independent of risk factors in multivariate regression analysis. In contrast, prevalence of coronary arterial calcification was similar between CGD patients and controls (14.6%, CGD, and 6.3%, controls, $p=0.39$).

***Conclusions*—**The observation by MRI of reduced carotid but not coronary artery atherosclerosis in CGD patients despite the high prevalence of traditional risk factors raises questions about the role of NOX2 in the pathogenesis of clinically significant atherosclerosis. Additional high-resolution studies in multiple vascular beds are required to address the therapeutic potential of NOX-inhibition in cardiovascular diseases.

***Clinical Trial Registration Information*—**clinicaltrials.gov. Identifier: NCT01063309.

Key words: atherosclerosis, inflammation, carotid artery, coronary, immune system

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Introduction

Chronic Granulomatous Disease (CGD) is an inherited immunodeficiency caused by mutations in genes encoding the main components of the phagocyte NADPH oxidase (NOX2) ,gp91 ^{phox}, p22 ^{phox}, p47 ^{phox}, p67 ^{phox}, and rarely p40 ^{phox}, resulting in impaired production of superoxide anion and other reactive oxygen intermediates.[1] Hemizygous mutations in gp91^{phox} cause the most common form, X-linked CGD (X-CGD), while autosomal CGD (A-CGD) is due to mutations in the other subunits. [2] CGD manifests clinically with recurrent infections and granulomatous complications. [3] Lower levels of residual ROS production by neutrophils are associated with earlier mortality. [2] Significantly elevated production of pro-inflammatory mediators by CGD myeloid cells (*e.g.,* IL-8 [4], LTB_4 [5]), and decreased neutrophil apoptosis [6] are also thought to contribute to the excessive inflammation secondary or independent of infection that is often seen in CGD.

Beyond a role in immune defense, increased inflammation with associated increased reactive oxygen species generated by a family of NOX proteins, NOX2, NOX1 and NOX4, have been implicated in the pathogenesis of cardiovascular disease and atherosclerosis. [7] NADPH oxidases contribute to the differentiation and migration of vascular smooth muscle cells, endothelial cell response to nitric oxide, and are highly expressed in atherosclerotic plaque.[8-12] Reduced NADPH oxidase activity may reduce vascular inflammation and thereby decrease susceptibility to atherosclerosis – a possibility that makes pharmacologic inhibition of NOX a potential target of therapy for cardiovascular diseases. [13]

Mouse models of NOX deficiency have yielded conflicting results on atherosclerosis progression. [14-18] Pharmacologic inhibition of NOX in murine models has succeeded in reducing atherosclerosis progression. [19] Studies in gp91 ^{phox} and p47 ^{phox} - deficient human CGD subjects

demonstrated significant differences in cardiovascular function. Enhanced arterial dilatation and vascular endothelial function following ischemia and reperfusion have been noted in CGD. [20, 21] Enhanced arterial dilatation was noted in male CGD patients and lower carotid intimal-medial thickness in X-CGD patients and female carriers compared to healthy subjects[20 22], suggesting that even a 50% reduction in NOX2 function is sufficient for cardiovascular protective effects. To date, no studies have reported the effects of NOX2 deficiency in the coronary circulation. We investigated markers of inflammation and the prevalence of subclinical carotid and coronary atherosclerosis using noninvasive MRI and CT techniques in normal controls and patients with gp91 *phox* or p47 *phox* CGD.

Methods

Patients

Patients over 18 years of age with a clinical diagnosis of CGD and either gp91 *phox* or p47 *phox* deficiency and healthy volunteers in the same age range were enrolled from 2010-2014 in an IRB approved protocol (10-I-0029) conducted at the NIH Clinical Center. All subjects provided documented informed consent. CGD diagnoses were confirmed by genetic sequencing and/or western blotting as well as quantitation of reactive oxygen species.[2] Volunteers underwent history and physical examination to confirm that they were free of clinical cardiovascular disease or active systemic infection. Patients with fever, atrial fibrillation or contraindication to gadolinium or MR imaging were excluded. Patients with contraindication to iodinated contrast were eligible to undergo MR and non-contrast CT (calcium scoring). No CGD patients in this study had received a bone marrow transplant.

Acquisition and Analysis of Carotid MR Imaging

Carotid wall volume was determined to assess the extent of atherosclerotic disease.[23] MR

imaging was performed on a 3 T clinical scanner (Verio, Siemens) using four-channel carotid coils (Machnet). T1 pre & post contrast and T2 weighted fat-suppressed, ECG-gated black blood images were obtained using double inversion recovery fast spin echo sequences. Post-contrast T1 weighted images were obtained 5 minutes after an intravenous dose of 0.1 mmol/kg gadopentetate dimeglumine (Magnevist, Bayer HealthCare). Scan resolution was 0.50 mm x 0.50 mm x 2.0 mm, with 5 consecutive slices and no gap obtained in each internal carotid artery (ICA) beginning at the carotid bifurcation.

ICA wall volume was quantified using QPlaque (version 1.0, Medis) by a blinded observer. The area within the external boundary of the vessel and the arterial lumen were semi-automatically contoured on post-contrast T1 images, using pre-contrast T1 and T2 weighted images to confirm vessel boundaries in slices with flow artifact. [24, 25] The corresponding volume was obtained by multiplying the area in each image by the slice thickness and summing the total number of slices obtained. Wall volume was calculated by subtracting lumen volume from total vessel volume.

Acquisition and Analysis of Cardiac CT Angiography

Coronary artery wall volume and calcium score were determined using CT angiography as measures of atherosclerotic disease.[26] Pre- and post-contrast CT imaging was performed using a 320-detector row scanner with slice thickness 0.5 mm (Aquilion ONE, Toshiba Medical Systems). Calcium scoring was performed with prospective ECG gating with 350 msec gantry rotation, 120 kV tube voltage and 300 mA tube current and quantified by the Agatston method.[27] CT angiography was performed after administration of intravenous iopamidol (Isovue 370, Bracco Diagnostics) using 120 kV tube voltage and tube current 350-580 mA dependent on BMI. Beta-blockers were administered if the resting heart rate was >60 beats per minute.

Image analysis was performed on a Vitrea FX workstation (Version 6.1, Vital Images). The left main coronary artery and proximal coronary artery segments were segmented according to previously published definitions.[28] The lumen and external vessel wall were semi-automatically contoured at 0.5 mm intervals. The area between the lumen and external wall was multiplied by slice thickness and summed over the total number of slices per segment to calculate coronary arterial wall volume (mm^3). Coronary artery wall volume was indexed to segment length to account for variability in coronary anatomy. The resulting value is reported as the coronary plaque index (mm^2). Inter-reader reproducibility for this method in a sub-sample of 10 consecutive participants was excellent (ICC=0.97).

Plasma Analytes

Glucose, lipids, and biomarker measurements were obtained after ≥ 12 hour fasting. Biomarker analysis was on plasma prepared from heparinized blood by 2 centrifugation steps at 500 g for 10 min. Plasma aliquots were stored in the vapor phase of liquid N_2 freezer prior to analysis. IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, IFN-γ, and TNF-α were measured with the TH1/TH2 Ultrasensitive 10-Plex (Meso Scale Discovery, Gaithersburg, MD) while IL-6, IL-17, GM-CSF, MIP-1α MIP-1β, RANTES, soluble TNF-RI, soluble TNF-RII, and soluble IL-6R were measured with customized multiplex cytokine immunoassays (Meso Scale Discovery) using a SECTOR™ Imager 6000 reader (Meso Scale Discovery). Standard curves were analyzed using nonlinear 4-parameter curve fitting and unknowns were calculated based on the best fit equation. For multiplex cytokine assays, an internal control was run on each plate to monitor inter-assay variability (mean CV% was 50.4%; range 16.3 – 111.4%). An aliquot of standard was spiked into a control plasma sample to determine the recovery of the specific cytokine standards in the sample matrix (mean recovery was 95.7%; range 78.2 – 119.8%). Commercial immunoassays

were used for: oxidized LDL (Mercodia, Uppsala, Sweden), matrix metallopeptidase 9 (R&D Biosystems, Minneapolis, MN), lactoferrin (Oxis International ,Portland, OR), α-defensins (Hycult Biotech, Plymouth Meeting, PA), and myeloperoxidase (Meso Scale Discovery). Plasma levels of total nitrate/nitrite (NO_x^-) and nitrite (NO_2^-) were determined using a Total Nitric Oxide and Nitrate/Nitrite kit (KGE001, R&D Systems, Minneapolis, MN).

Statistical Analysis

Continuous outcomes were summarized using means and standard deviations (SDs) or medians and interquartile ranges (IQRs) as appropriate, while counts and percentage values were reported for binary outcomes. Continuous outcomes of different subgroups were compared using t-tests with unequal variances. Binary outcomes were compared by chi-squared test. Spearman's correlation coefficient was used to summarize the correlation between two continuous outcomes. Univariable and multivariable linear regression models were used to evaluate the unadjusted and adjusted effects of potential risk factors on carotid wall volume and coronary plaque burden. Two multivariable linear regression models were constructed: a base model adjusted for age and gender, and a second model including traditional clinical cardiovascular risk factors. Coefficients of determination R^2 were reported to summarize the predictive power of a regression model. Carotid wall volume measures were logarithmically transformed (on a common log scale) before analysis to reduce skewness. Data analyses were carried out using STATA 10.1 (College Station, TX). All p values were two-sided and were not adjusted for multiple comparisons given the exploratory nature of this study. Graphpad Prism version 6.0d was used for ANOVA with a Holm-Šídák post-test [29]. P values less than 0.05 were considered statistically significant.

Results

Patients

Twenty-two CGD patients with mutations in gp91^{phox}, 19 patients with mutations in p47^{phox} and 25 age- matched healthy controls were enrolled. Demographic and clinical characteristics of the study population are shown in **Table 1**. CGD patients were 32.5 ± 9.4 years old and 76% were male. CGD patients had significantly elevated cardiovascular risk factors compared with controls, including more prevalent hypertension, decreased HDL-c, as well as increased oxidized LDL. Amongst CGD patients, those with p47^{phox} mutations had significantly greater residual superoxide production than those with gp91^{phox} mutations (3.0 ± 0.7 vs. 1.7 ± 1.8 nM superoxide/ 1 x 10^6 neutrophils/ 60 min respectively, $p<0.01$), about 1.3 % and 0.7% of superoxide produced by neutrophils from normal volunteers (226.29 ± 3.01 nM superoxide/1 x 10^6 neutrophils/60 min).[2]

Plasma markers of inflammation in CGD

CGD subjects had higher levels of classical acute phase reactants (hsCRP) and innate defense proteins (myeloperoxidase, lactoferrin, and α-defensins) suggesting increased neutrophil degranulation and systemic inflammation. Pro-inflammatory cytokines such as IL-6 and TNFα as well as the chemokines CXCL8 (IL-8) and CCL4 (MIP1β) were significantly elevated in CGD (**Table 1**).

Subclinical Atherosclerosis

Internal Carotid Artery

Patients with impaired NADPH oxidase function had 22% lower internal carotid arterial (ICA) wall volume than age-matched healthy individuals. The mean ICA wall volume in CGD patients was 361.3 ± 76.4 mm^3 , compared with 463.5 ± 104.7 mm^3 in controls ($p<0.001$. To reduce

skewness the data were log-transformed prior to the statistical analyses that follow. The mean ± standard deviation of the common log-transformed ICA wall volume in CGD patients was 2.55 ± 0.02 $\log_{10}$ mm^3, compared with 2.66 ± 0.02 $\log_{10}$ mm^3 in controls (p=0.0001, **Table 2** & **Figure 1A**). No participant in either group had evidence of atherosclerotic plaque that resulted in significant carotid luminal stenosis.

There were significant univariate associations between ICA wall volume and CGD, HDL-c, IL-6, IL-10, IL-12p70 and IL-13. (**Table 3**) Only CGD (regression coefficient, -0.14; 95% CI, -0.21 – -0.08; p<0.001) male gender (regression coefficient, 0.06; 95% CI, 0.01 – 0.12; p=0.02) and hypertension (regression coefficient, 0.09; 95% CI, 0.02 – 0.15; p=0.01) remained as significant predictors of ICA wall volume after controlling for traditional cardiovascular risk factors in a multivariate linear regression model (R^2 = 0.50).

Coronary Plaque Index and Coronary Arterial Calcium (CAC)

Cardiac CT angiography and calcium scoring were performed in 21 CGD patients and 16 healthy volunteers. Analyzable results were obtained in 36 of 37 scans (97.3%). Four CGD patients and one volunteer had atherosclerotic plaque resulting in <50% luminal stenosis. One CGD patient and no volunteers had plaque resulting in >50% luminal stenosis. The coronary plaque index, including calcified and non-calcified plaque, was similar in CGD patients and controls (8.3 ± 2.1 and 7.8 ± 1.5 mm^2 respectively, p=0.46).

Prevalent coronary calcification, defined as an Agatston score ≥ 1, was present in one healthy control and 6 CGD patients (6.3% and 14.6%, respectively, p=0.39). The median CAC in CGD patients with measurable calcification was 173 (IQR 9-366) and CAC for the control patient was 5.

Significant univariable associations with coronary plaque were present for age and

hypertension but not CGD. (**Table 4**) A minimally adjusted multivariable linear regression model including CGD, age and gender found significant associations between age and gender – but not CGD – with coronary plaque. After correction for CGD and traditional cardiovascular risk factors in a multivariable linear regression model, only hypertension emerged as an independent predictor of coronary wall volume (coefficient of association=2.3; 95% CI, 0.5 – 4.1; p=0.02; overall r^2 for model 0.52, p<0.01). ICA wall volume and coronary arterial wall volume were not significantly correlated (Spearman's rho = 0.18, p=0.33).

Subgroup Analyses

Amongst patients with CGD, those with deficiency in gp91 ^{phox} (X-linked CGD) were, as expected, entirely male while the p47 ^{phox} patients were evenly divided between men and women (47% male). TNF-α levels were significantly lower in p47 ^{phox} patients. We otherwise observed no significant differences in prevalent traditional cardiovascular risk factors, lipid subfraction levels, or markers of systemic inflammation by genotype. (**Table 5**)

As expected, in healthy control subjects the carotid artery wall volume was significantly lower in females compared with males (**Fig 1B**). CGD gp91 ^{phox} deficient patients (all male) had significantly reduced carotid artery wall volume compared with age matched control males (**Figure 1B**). CGD p47 ^{phox} deficient male and female patients also had significantly reduced carotid artery wall volume compared with age and sex matched controls (**Figure 1B**). There was no significant relationship between residual superoxide production and the internal carotid artery wall volume among all the CGD patients (not shown) nor was there a significant difference between common log-transformed internal carotid artery wall volume of p47 ^{phox} deficient and gp91 ^{phox} deficient patients (2.53 ± 0.02 vs. 2.57 ± 0.02 $\log_{10}$ mm^3, p=0.19).

There was no difference between control subjects and CGD patients in coronary arterial

plaque burden (p47 ^{phox}, 8.4 ± 2.5 vs. gp91 ^{phox} 8.1± 1.4 mm^2, p=0.75) or the prevalence of coronary arterial calcium (p47 ^{phox} 13.6% vs. gp91 ^{phox} 15.8% p=0.85).

As intraconazole has been demonstrated to affect HDL levels [30] we conducted a sensitivity analysis excluding 16 patients on active itraconazole prophylaxis therapy. The remaining 20 CGD patients still had a significant reduction in carotid atherosclerosis compared to healthy controls (p=0.0012).

Discussion

We investigated the prevalence of subclinical atherosclerosis in the carotid and coronary arteries of patients with CGD. Despite an adverse cardiovascular risk profile with significant elevations in multiple systemic markers of inflammation, the data demonstrate that CGD was associated with smaller carotid artery wall thickness, an established indicator of subclinical atherosclerosis, compared to healthy controls. This effect was independent of traditional cardiovascular risk factors. In contrast, CT angiography of coronary arteries did not show a significant difference between CGD and control subjects in the prevalence of coronary atherosclerosis.

CGD offers an opportunity to study the clinical consequences of reduced NOX2 activity on cardiovascular disease in humans. Violi and coauthors found a reduction in ultrasound carotid intimal-medial thickness in male patients with CGD [20] and in female carriers of X-linked CGD.[22] While X-CGD carriers are generally healthy, many face an increased frequency of autoimmune disease [31] and, depending on the extent of lyonization in their myeloid cells, some face serious CGD-like infectious complications. [32] Nevertheless, a reduction in carotid intimal-medial thickness in both patients and carriers was associated with lower biomarkers of oxidative stress and increased brachial arterial flow mediated dilation. These investigators also reported

decreased isoprostane formation and increased NO generation in X-CGD.[20] Increased flow mediated dilation was reported in p47 *phox* -deficient CGD patients but intimal-medial thickness did not differ from normal.[33] These results provided the first evidence *in vivo* that NOX2 may play a role in arterial tone and hypertension, and possibly contribute to the pathogenesis of atherosclerotic disease.[20, 22, 33] Although intimal-medial thickness is considered a useful surrogate marker for atherosclerosis, hypertension alone is a primary driver of intimal thickening, and can cause changes in carotid intimal-medial thickness that do not correspond histologically to atherosclerotic injury.[34] It is plausible that the observed reduction in carotid intimal-medial thickness was driven by NOX2 related improvement in endothelial function in a process independent of atherosclerosis. Flow mediated dilation provides useful insights into arterial endothelial function, but has not been demonstrated to predict future cardiovascular events beyond traditional risk factors.[35] The correlation between carotid and coronary atherosclerotic burden, while significant in some populations, has important limitations [36] , and it remains possible that the observed reduction in carotid intimal-medial thickness may not translate into a protective effect in other vascular beds consistent with previous observations in a mouse model. [37] While associations between morphologic carotid atherosclerosis and coronary arterial disease [38, 39] incident cardiovascular events have been observed, the presence and strength of these correlations varies importantly by population. [36]

The present study demonstrates an association between both p47 *phox* and gp91 *phox* CGD and lower carotid wall thickness using histologically validated high-resolution MRI techniques. [25, 40] Our analysis shows this difference in early vascular injury is independent of hypertension, suggesting that it may be mediated through other NOX2 effects as discussed below. Importantly, p47 *phox* is not only a cytosolic regulator of NOX2 activity but can also regulate NOX1. [41]

Deficiency in this protein may, therefore, alter multiple NADPH oxidases.

This is the first study, to our knowledge, to examine the prevalence of subclinical coronary atherosclerosis in CGD. In contrast to the findings in the carotid arteries, noninvasive quantification of wall thickening and coronary plaque burden showed no significant difference between NOX2-deficient patients and controls. Quantification of coronary arterial calcium, a finding representative of more advanced atherosclerotic lesions and a powerful independent predictor of cardiovascular events, [35, 42] showed no reduction and a trend towards a greater burden of calcified atherosclerosis in CGD patients. Physiologically, it is possible that NOX2-related mechanisms play a lesser role in coronary atherosclerosis than in other arterial beds. While there was a non-statistically significant trend towards greater coronary calcification in CGD patients, the total volume of calcified and noncalcified plaque, as measured by coronary plaque index, was nearly identical to controls. The ratio of calcified to non-calcified components of atherosclerotic plaque varies in different disease processes, [43-45] and patients with CGD may have more rapid progression of calcification than found in typical atherosclerosis. We cannot exclude the possibility that small sample size limited the power to detect a smaller magnitude difference in coronary plaque burden between groups. Given the increases in the lifespan of CGD patients since the advent of antimicrobial prophylaxis, further investigation of the aging CGD population will likely reveal whether ROS play a role in calcified coronary atherosclerosis.

Our study of CGD patients also revealed significantly lower levels of HDL and a trend toward elevated triglycerides despite normal cholesterol. This is in contrast to the reported decrease in triglycerides in CGD mice. [14] Surprisingly, oxidized LDL was significantly higher in CGD suggesting not only that NOX2 is not primarily responsible for the production of oxidized LDL but possibly that NOX2 is involved in the catabolism of this lipid species. Importantly,

CGD subjects are treated with prophylactic antibacterial and antifungal drugs including itraconazole, which was been shown to reduce LDL and increase HDL in immunocompetent men. [30] We did not detect any association between itraconazole prophylaxis or other antibiotic therapies our patients were receiving and atherosclerotic burden or lipid profiles.

Despite the absence of overt signs of infection including normal white cell counts, plasma from CGD subjects in this study contained significantly increased concentrations of clinically recognized cardiovascular risk factors such as hsCRP [46] and MPO [47] as well as other inflammatory biomarkers (*e.g.*, TNFα, IL-6, GM-CSF). These elevations, which have not been reported previously in CGD, may be due to unrecognized infection or inflammation but may also relate to the direct regulatory role for reactive oxygen species in controlling inflammation. For example, ROS regulates the mRNA stability of IL8 [4], the metabolism of leukotrienes resulting in accumulation of LTB_4 [5], IL-1β processing [48] and the apoptosis of CGD neutrophils [6]. The role of cytokines as regulators of inflammation in cardiovascular disease has been reviewed [49] although specific pathophysiologic roles and clinical risk guidelines have yet to be established. Interestingly, a common polymorphism in the IL-6 receptor that reduces function was associated in an 82-study meta-analysis with a decreased risk of coronary heart disease suggesting that signaling by IL-6, which was also elevated in CGD, may be pathogenic.[50] In parallel with MPO, we also observed significant elevations in CGD patient plasma of other neutrophil-derived factors including gelatinase, lactoferrin and defensins. Further work will be required to determine whether or not the increases in these factors are due to mechanisms such as those controlling IL-8 (see above) or reflect neutrophil degranulation due to increased inflammation in CGD.

The observation of reduced carotid but not coronary artery atherosclerosis in CGD patients raises questions about the role of NOX2 in the pathogenesis of atherosclerosis.

Importantly, this finding also raises questions about whether or not NOX2 inhibitors may be beneficial in reducing all atherosclerosis or just that outside of the coronary circulation. Clearly, further high-resolution studies of possible links between deficiencies in or inhibition of different NOX proteins and atherogenesis in distinct anatomic vascular locations are indicated.

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Table 1. Clinical and biomarker characteristics of study population.

		CGD (n=41)	Control (n=25)
Clinical Characteristics	Age	32.5±9.3	32.1±7.1
	Male, N (%)*	31 (75.6%)	12 (48.0%)
	Hypertension*	12 (29.3%)	1(4.0%)
	Diabetes	3 (7.3%)	0 (0%)
	Smoking	14 (34.2%)	7 (28.0%)
	Family CVD history*	22 (56.4%)	6 (24.0%)
Lipid Levels	Total Cholesterol (mg/dL)	153.2±35.0	162.4±28.1
	LDL (mg/dL)	92.7±33.9	84.7±21.7
	HDL (mg/dL) ***	38.1±10.9	60.2±14.7
	Triglycerides (mg/dL)	110.6±68.2	87.8±50.9
	Oxidized LDL (mg/dL) ***	65.0±37.2	23.4±18.6
Other Biomarkers	hsCRP (mg/L) ***	8.9±10.1	2.4±3.8
	Nitrite (NO_2^-, μM)	2.6±1.5	2.8±1.0
	Nitrate (NO_3^-, μM)	24.7±16.8	22.8±17.6
Neutrophil proteins	Lactoferrin (ng/ml) ***	722.6±832.0	225.0±98.3
	MPO (ng/ml) **	473.0±810.7	118.4±76.3
	MMP-9 (ng/ml) ***	375.7±420.4	134.5±63.9
	α-Defensins (ng/ml)*	56.3±137.1	8.1±5.3
Cytokines	IFN-γ (pg/ml)	5.5±16.1	1.8±6.0
	TNF-α(pg/ml)**	10.7±5.7	4.7±7.4
	GM-CSF (pg/ml)*	3.1±6.8	0.7±0.7
	IL-1β(pg/ml)	1.2±4.4	0.4±0.5
	IL-2 (pg/ml)	0.8±0.8	1.2±2.5
	IL-4 (pg/ml)	0.2±0.5	0.3±0.6
	IL-5 (pg/ml)	1.8±6.1	2.3±7.1
	IL-6 (pg/ml)**	10.3±11.0	4.4±5.5
	IL-10 (pg/ml)	3.9±8.0	10.3±37.4
	IL-12p70 (pg/ml)	10.0±56.7	31.9±146.9
	IL-13 (pg/ml)	2.2±4.4	45.5±184.5
	IL-17 (pg/ml)	12.6±19.1	7.6±7.8
Chemokines	CCL3 (MIP-1α, pg/ml)	38.8±42.2	27.7±13.7
	CCL4 (MIP-1β, pg/ml)**	178.7±226.5	60.2±39.0
	CCL5 (RANTES, ng/ml)	25.9±19.2	25.9±13. 3
	CXCL8 (IL-8, pg/ml)*	19.6±37.6	5.5±6.6
Soluble Selectins	sL-selectin (ng/ml)	1,163.9±245.6	1,152.5±187.5
	sE-selectin (ng/ml)*	39.8 ± 17.4	31.3±13.8
Soluble Receptors	TNFR1 (pg/ml)	3,376.0±2434.9	2,914.8±1,256.7
	TNFR2 (pg/ml) ***	3,986.5±2,170.6	2,389.4±652.2
	IL-6R (pg/ml)	18,223.8±4,780.8	18,695.1±5,162.2

Means ± SD. * denotes $P \leq 0.05$, ** denotes $P \leq 0.01$, and *** denotes $P \leq 0.001$ for difference between CGD and control patients respectively. Abbreviations: CGD, chronic granulomatous disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hsCRP, high sensitivity C-reactive protein; ESR, erythrocyte sedimentation rate; MPO, myeloperoxidase; IL, interleukin; TNF, tumor necrosis factor; GM-CSF, granulocyte macrophage colony stimulating factor; MIP, macrophage inflammatory protein; MMP, matrix metallopeptidase

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Table 2. Markers of subclinical atherosclerosis.

	Control	CGD	p-value
Internal carotid arterial wall volume (log_{10} mm^3)	2.66± 0.02	2.55 ± 0.02	0.0001
Prevalent coronary arterial calcium (n,%)	1 (6.3)	6 (14.6)	0.39
Coronary plaque index (mm^2)	7.8 ± 1.5	8.3 ± 2.1	0.46

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Table 3. Internal Carotid Arterial Wall Volume in Univariate and Multivariable Linear Regression Analyses.

	Univariate Model			Multivariable base model (R^2 = 0.38)			Multivariable model 2 (R^2 = 0.50)		
	coeff.	SE	p-value	coeff.	SE	p-value	coeff.	SE	p-value
CGD	**-0.11**	**0.03**	**<0.001**	-0.13	0.02	<0.001	-0.14	0.03	<0.001
age	0.001	0.002	0.56	0.002	0.001	0.23	0.001	0.001	0.70
male gender	0.05	0.03	0.09	0.08	0.02	0.002	0.06	0.03	0.02
hypertension	0.03	0.03	0.36				0.09	0.03	0.01
smoking	-0.02	0.03	0.48				-0.002	0.03	0.94
family history	-0.01	0.03	0.81				0.04	0.03	0.09
LDL	0.08×10^{-3}	0.48×10^{-3}	0.87				0.05×10^{-3}	0.4×10^{-3}	0.90
HDL	2.0×10^{-3}	0.8×10^{-3}	0.02				1.0×10^{-3}	1.0×10^{-3}	0.31
hsCRP	-0.0×10^{-3}	1.5×10^{-3}	0.55						
oxidized LDL	-0.6×10^{-3}	0.4×10^{-3}	0.11						
lactoferrin	-26×10^{-6}	19×10^{-6}	0.17						
MPO	-39×10^{-6}	20×10^{-6}	0.053						
MMP-9	-32×10^{-6}	39×10^{-6}	0.40						
TNF-α	-0.2×10^{-6}	0.1×10^{-6}	0.08						
GM-CSF	1.6×10^{-3}	3.4×10^{-3}	0.64						
IL-1β	-2.2×10^{-3}	3.9×10^{-3}	0.57						
IL-6	3.4×10^{-3}	1.3×10^{-3}	0.01						
IL-10	1.7×10^{-3}	0.5×10^{-3}	0.002						
IL-12p70	0.4×10^{-3}	0.1×10^{-3}	0.002						
IL-13	0.3×10^{-3}	0.1×10^{-3}	0.002						
IL-17	0.5×10^{-3}	0.8×10^{-3}	0.58						
CCL3 (MIP-1α)	-0.3×10^{-3}	0.4×10^{-3}	0.43						
CCL4 (MIP-1β)	0.4×10^{-6}	72×10^{-6}	0.99						
CCL5 RANTES	-0.7×10^{-6}	0.8×10^{-6}	0.37						
CXCL8 (IL-8)	-0.3×10^{-3}	0.4×10^{-3}	0.50						
TNFR1	4×10^{-6}	7×10^{-6}	0.54						
TNFR2	-8×10^{-6}	8×10^{-6}	0.29						
IL-6R	2×10^{-6}	3×10^{-6}	0.43						

Coeff is the estimated regression coefficient. SE is the variance of the estimated regression coefficient.

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Table 4. Coronary Plaque Index in Univariable and Multivariable Linear Regression Analyses

	Univariate Model			Multivariate Model 1 ($R^2 = 0.28$)			Multivariate Model 2 ($R^2 = 0.52$)		
	coeff.	SE	p-value	coeff.	SE	p-value	coeff.	SE	p-value
CGD	0.44	0.61	0.47	0.14	0.55	0.80	-0.33	0.69	0.64
age	**0.09**	**0.03**	**0.01**	**0.09**	**0.03**	**0.007**	0.06	0.04	0.11
male gender	1.10	0.61	0.08	**1.23**	**0.57**	**0.04**	0.52	0.63	0.42
hypertension	**3.2**	**0.69**	**<0.001**				**2.29**	**0.89**	**0.02**
smoking	-0.15	0.68	0.83				-0.21	0.59	0.72
family history	-0.10	0.61	0.87				-0.08	0.61	0.90
LDL	-0.01	0.01	0.47				-0.02	0.01	0.08
HDL	-0.03	0.02	0.09				-0.02	0.02	0.31
hsCRP	0.03	0.03	0.38						
oxidized LDL	0.01	0.01	0.26						
MPO	-0.0006	0.0005	0.24						

Coeff is the estimated regression coefficient. SE is the variance of the estimated regression coefficient.

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Table 5. Clinical and biomarker characteristics of CGD patients by genotype.

		p47 (n=19)	gp91 (n=22)
Clinical Characteristics	Age	34.8±8.0	30.4±10.1
	Male, N (%)***	9 (47.4%)	22 (100%)
	Hypertension	5 (26.3%)	7 (31.8%)
	Diabetes	3 (15.8%)	0 (0%)
	Smoking	8 (42.1%)	6 (27.3%)
	Family CVD history	13 (68.4%)	9 (45.0%)
Lipid Levels	Total Cholesterol (mg/dL)	149.1 ± 24.4	156.8 ± 42.7
	LDL (mg/dL)	85.9 ± 25.1	99.2 ± 40.1
	HDL (mg/dL)	41.4 ±12.0	35.2 ±9.2
	Triglycerides (mg/dL)	108.6 ± 57.1	112.3 ± 78.4
	Oxidized LDL (mg/dL)	57.4 ± 36.8	71.6 ± 37.1
Other Biomarkers	hsCRP (mg/L)	7.5 ± 9.4	10.1 ± 10.8
	Nitrite (NO_2^-, μM)	2.8±1.4	2.5±1.5
	Nitrate (NO_3^-, μM)	21.3±12.7	27.5±19.6
Neutrophil proteins	Lactoferrin (ng/ml)	538.9 ± 708.7	881.3 ± 911.7
	MPO (ng/ml)	442.1 ± 819.7	499.7 ± 821.2
	MMP-9 (ng/ml)	248.2 ± 252.2	485.8± 504.7
	α-Defensins (ng/ml)	63.3 ± 187. 9	50.2 ± 74.0
Cytokines	IFN-γ (pg/ml)	2.1 ± 2.0	8.4 ± 21.7
	TNF-α(pg/ml)	8.6 ± 4.2*	12.5 ± 6.2
	GM-CSF (pg/ml)	1.4± 2.2	4.6 ± 8.9
	IL-1β(pg/ml)	0.6 ±0.7	1.6 ± 5.9
	IL-2 (pg/ml)	0.8 ±0.9	0.8 ±0.6
	IL-4 (pg/ml)	0.3 ±0.6	0.1 ±0.3
	IL-5 (pg/ml)	0.8 ±0.6	2.6 ±8.3
	IL-6 (pg/ml)	11.1± 14.1	9.6 ± 7.8
	IL-10 (pg/ml)	2.9 ± 1.8	4.8 ± 10.8
	IL-12p70 (pg/ml)	1.2 ± 1.1	17.6 ± 77.4
	IL-13 (pg/ml)	1.7 ± 3.5	2.5 ± 5.1
	IL-17 (pg/ml)	11.0± 11.4	13.9 ± 24.1
Chemokines	CCL3 (MIP-1α, pg/ml)	39.2 ± 48.5	38.3 ± 37.2
	CCL4 (MIP-1β, pg/ml)	128.9 ± 66.5	221.8 ± 299.5
	CCL5 (RANTES, ng/ml)	23.7± 9.7	27.9 ± 24.8
	CXCL8 (IL-8, pg/ml)	13.9 ± 24.6	24.6 ± 46.0
Soluble Selectins	sL-selectin (ng/ml)	1108.4 ± 218.5	1211.8 ± 262.2
	sE-selectin (ng/ml)	38.5 ± 15.3	41.0± 19.3
Soluble Receptors	TNFR1 (pg/ml)	3057.0 ± 1373.8	3651.6 ± 3082.7
	TNFR2 (pg/ml)	3514.7± 1204.8	4393.9 ± 2712.0
	IL-6R (pg/ml)	17576.0 ± 3229.0	18783.2 ± 5821.6

Means ± SD. * denotes P ≤ 0.05, ** denotes P ≤ 0.01, and *** denotes P ≤ 0.001 for difference between CGD patients with deficiency $p47^{phox}$ and $gp91^{phox}$ deficiency.

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Figure Legend:

Figure 1. Comparison of Subclinical Coronary and Carotid Atherosclerosis in Healthy Controls and CGD Patients. Bars denote mean and 95% CI of the mean and p-values are from t-tests (Panels A, C) and ANOVA with a Holm-Šídák post-test (Panel B).